Chapter 3

Varietal differences in physico-chemical and phytochemical properties of pigmented rice

3.1. Introduction

Rice (*Oryza sativa* L.) is considered as a staple food in South East Asia and is the most popular cereal crop, consumed by over half of the world population [44]. Pigmented rice grain varied from deep-purple (black) to brown-reddish (red) in their covering layers, due to the accumulation of natural pigments in the seed coat called anthocyanin, water soluble flavonoids. It has a typical nutty flavour, aroma which turns into deep dark purple when cooked. It is stickier than the white rice after cooking and takes longer time to cook. Such pigmented rice is commonly known as 'Chak-hao' in Manipur, India, meaning 'delicious rice' (Chak = rice and ahaoba or hao = delicious). The major contributor of aroma is due to the presence of volatile compound 2-acetyl-1-pyrroline which was first isolated and identified by Buttery et al., [4]. Chakhao Poreiton (purple) and Chakhao Amubi (black) are two main aromatic rice varieties grown in Manipur.

Pigmented rice is a main source of various bioactive compounds which provide anti-inflammatory properties, anti-diabetic and also inhibit new blood vessels formation which enhances tumour growth [22]. This rice also contains many phytochemicals and having a beneficial effect on health. The whole grains are major resources of various biologically active components such as phenolic acids, antioxidants, vitamins, phytates, tocopherols, and carotenoids and due to these multiple biological activities of black and other pigmented rice varieties, there has been an increase in demand. Anthocyanin is one of the primary functional ingredients of pigmented rice [9], obtained from the 2- phenyl benzopyrilium or flavylium ion. Again, phenolic acids are present in both free and bound form. The extracts of black rice bran provided inhibitory effects on *in vitro* allergic reactions [6]. Pigmented rice bran (black and red) extracts inhibit α -glucosidase and α-amylase activity which also help in delaying of digestion and absorption of carbohydrates that leads to suppressing the postprandial hyperglycaemia in the diabetic person. Interests has been given by the consumers due to the nutrient content of these rice varieties for utilization in nutraceuticals and has enticed the food industries. Although the phytochemicals available in the whole rice kernels are minute quantity, however, provide

various benefits to health in minimizing the risks of many diseases such as cardiovascular and heart diseases, obesity, type II diabetes and some types of cancer [36]. Again, the rice bran is an underutilized coproduct produced in the process of milling. During the last decades, research conducted has reported that the rice grain provides a complex unique antioxidant compound occurring naturally [26].

Quality parameters such as grain shape and size, grain appearance, cooking and eating qualities play a critical role in consumer preference as rice is consumed as a whole grain [27]. Grain quality comprises a number of traits such as physical appearance, cooking, eating, sensory properties and nutritional value [11]. Many studies have reported its importance in terms of nutritional and medicinal aspects, physicochemical, textural and rheological properties rice and its flour [5, 30, 39, 41]. Moreover, total phenolic content and antioxidant activity of colored and Thailand white rice varieties were determined using Fourier transform infrared (FT-IR) spectroscopy (400 to 4,000 cm^{-1}) as an effective alternative technique; for exploration of possibility of using FT-IR [16]. However, less study has been reported on the phytochemical properties of pigmented rice [12]. Nowadays, attention is being given to the anti-oxidative and radical scavenging properties of pigmented rice as their consumption encourages and promotes human health by minimizing the reactive oxygen species and free radical concentration. Again, it is estimated to found approximately 400 naturally occurring anthocyanins from the plant origin [2]. These anthocyanins, a polyphenol are also helpful for heart health [23]. However, the pigmented rice produced in Manipur is currently having limited use. It is consumed locally as steamed rice and is used for the preparation of sweets and very few ready to eat products. However, these are not explored in ready to eat commercial food products.

More research regarding its nutritional properties needs to be studied and explored. Even though there is high production of pigmented rice, and are also known for their antioxidant properties that provide many health benefits, there is limited study in terms of extraction, isolation and their utilization in food product development, fortification, formulation and value addition within a food matrix in order to utilized its medicinal, nutritional values in a better way for human health. Considering these aspects, study was planned to carry out its physico-chemical, cooking, pasting and phytochemical and antioxidant properties of locally grown pigmented rice of Manipur, India. The research plan was to develop functional foods from the best pigmented rice variety among the four considering the

antioxidant properties for its effective value addition. This study will be helpful for analyzing the nutritional properties, its deformation pattern involving in unit operations for further researches.

3.2. Material and methods

3.2.1. Materials

The research materials selected were three colored rice namely, Chakhao Poreiton (purple), Chakhao Amubi (black), Chakhao Angangba (red) and a white rice Chakhao Angouba (white) varieties, which is procured (5 kg each rice variety) from the city market (grown in Kakching district) of Manipur, India. The whole rice kernels were separated from broken rice for the evaluation of all the properties. The colored rice varieties were polished using rice polisher (NF366, Marathon, India) for the removal of bran for analysing its phytochemical properties. The rice samples were stored under refrigerated condition (4 ºC) for analysis purposes so that it does not get rancid.

3.2.2. Proximate analysis

All the rice varieties were analyzed for moisture, ash, fat and protein according to the standard procedures [21]. About 5 g each rice variety were dried in the hot air circulating oven at 105 ˚C to constant weight. The change in weight per amount of rice taken for analysis was used to determine the moisture content. Ash content in rice sample was estimated by loading the sample in muffle furnace (SNOL 8,2/1100, Lithuania) at 550 ºC for 6 h. The weight of ash generated per amount of rice taken was used for calculating the ash content.

Fat in the samples were determined using Socs plus (SCS6) apparatus (Pelican Equipment, Chennai, India). About 2 g of ground samples were weighed into tared cellulose thimbles and the top was plugged with fat-free cotton, and the thimble was attached to a Soxhlet flask. About 75-100 mL of n-hexane was poured through the sample. The condenser was attached to the top of the fat extraction tube. The sample was extracted for 8 h (boiling range 68-70 ºC) and thimble is removed. The flask containing oil and some solvent was heated at 80 ºC for 1 h for solvent removal. It was then cooled, weighed and amount of fat extracted per amount of rice taken for analysis was determined [28].

For the estimation of fiber content, the defatted residue of rice sample (2 g) was transferred into sintered glass crucible and attached to a Fibra plus apparatus (FES06, Pelican Equipment, Chennai, India. The sample was boiled with 200 mL of dilute H_2SO_4 solution (1.25 %) for 45 min. The acid was then drained off and washed with distilled water. Further, boiling with 200 mL of NaOH solution (1.25 %) for 45 min was done. The alkali was then drained off and washed with distilled water. The sample was dried for 2 h at 130 ˚C and cooled in a desiccator and weighed. Igniting was done for 2 h at 600 ˚C. Cooling was done and reweighed and the crude fiber content was determined [28].

Protein in rice sample was estimates using Kjeldahl method. 0.5 g of rice sample was taken in a Kjeldahl flask and 3 g catalyst of K_2SO_4: Cu_2SO_4 in the ratio 5:1 was added. The mixture was boiled with 15 mL conc. H_2SO_4 and digested until oxidation was complete (2 h). The content was then cooled and addition of 50 mL of water was done and mixed well. Addition of 50 mL of 40 % NaOH solution was done. It was then distilled until ammonia passed over into 4 % boric acid solution taken in a conical flask added with 2 drops of mixed indicator (methyl red + bromocresol green). Titration was carried out with standard 0.1 N HCl solution until the color changed from green to purple. The titre value was used to determine the total nitrogen content using the conversion factor 5.95. The total carbohydrate contents were determined by differences method.

3.2.3. Physico-chemical properties

The physical properties such as 1000 kernel weight, bulk density and length/breadth ratio of all the rice varieties were calculated [10]. For thousand kernel weight, milled head rice kernels from each rice varieties were randomly counted to thousand and the weight was recorded in grams. The bulk density was estimated by pouring the milled rice kernels into 100 mL graduated cylinder to a known volume of fixed height. A sample mass of the volume occupied was measured for all varieties and the ratio as g/mL was determined. The average length and breadth were measured using Rice Scanner (CanoScan 9000F Mark 11) machine for all rice varieties and the ratio (*L/B*) was calculated. The measurement was done in triplicate and reported.

Total amylose content for each rice varieties were estimated according to the method of [33]. 100 mg for each ground rice samples were dispersed in 1 mL of ethanol taken in a 100 mL volumetric flask, further addition of 10 mL of NaOH solution (4 g per 100 mL of distilled water) was done and left overnight. After incubation at room temperature, the

dispersion volume was made up to 100 mL with distilled water. 2.5 mL was taken in a 50 mL volumetric flask from this extract and titration was carried out against 0.1 N HCl followed by addition of 20 mL of distilled water and 3 drops of phenolphthalein indicator (which turned pink) showing the presence of amylose. Then 1 mL (0.2 %) iodine reagent was added and volume was made up to 50 mL and read the colour using UV-Visible Spectrophotometer at 590 nm (Spectronic 20D+, Thermo Scientific, USA). Amylose content was calculated from the standard curve for pure amylose solution at 20–100 µg/mL.

3.2.4. Phytochemical properties of rice and its bran

3.2.4.1. Extract preparation

The extraction process was carried out with some modification as described by Mir et al., [25]. 10 g of rice flours were treated with acidified ethanol (0.1 N HCl) of 50 mL for 1 h by stirring in an orbital shaker. Again, the rice:solvent ratio is one factor responsible for solutes diffusion in the extraction system where mass transfer and practical equilibrium takes place with thermal and non-thermal heating process [14]. Same extraction procedure was performed for all the rice bran varieties. The supernatant was collected after centrifugation of the solution was done at 1500 rpm for 30 min. The process of extraction was performed twice. The supernatant thus obtained were stored under refrigeration condition at 4^{o} C for further analysis of phytochemical and antioxidant content.

3.2.4.2. Total phenolic content (TPC)

Total phenolic content of the extract was determined by the modified Folin–Ciocalteu method [31]. Briefly, each extract of 200 µl was pipetted out in a test tube and followed by addition of 1 mL of FCR (Folin-Ciocalteu reagent) diluted to 1:10 with distilled water. The dispersion was shaken vigorously and addition of 1 mL of 10 % Na_2CO_3 solution was done and the solution was stirred and diluted it with distilled water to a final volume of 5 mL. The dispersed solution was kept to stand in the dark at room temperature for 2 h and the absorbance was measured at 765 nm using a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). Gallic acid (5 mg/ 100 mL) was used for measurement of standard. The calibration curve is generated by plotting concentration of the standard (0.2, 0.4, 0.6, 0.8 and 1 mL of gallic acid) along X-axis and the corresponding absorbance values at 765 nm along Y-axis; resulting a straight line.

3.2.4.3. Total flavonoid content (TFC)

Total flavonoid contents of each extract from rice were analyzed with some modification as described by Mir et al., [25]. The extracts (250 µl) were diluted with distilled water of 1.25 mL. Then, 75 µL of 5% $NaNO_2$ solution was added on it. The dispersion was kept to stand in the dark at room temperature for 6 min and 150 µl of 10 % $AlCl_3$ solution was added. Again, the solutions were kept to stand for 5 min and 0.5 mL of 1 M NaOH solution was added. Further, for dilution of the solution, 3 mL with distilled water was added and shaken vigorously and the absorbance was noted immediately at 510 nm using a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). Quercetin (5 mg/ 100 mL) was used as standard. Quantification was expressed by reporting the absorbance in the calibration graph of quercetin. The calibration curve was obtained using the concentrations 0.2, 0.4, 0.6, 0.8 and 1 mL of quercetin vs corresponding absorbance. Concentration values of the rice bran extract were obtained from the standard curve, by interpolating to the X-axis. TFC was calculated by using Eq. (3.1).

$$\text{TFC} = \frac{R \times D.F \times V \times 100}{W} \qquad (3.1)$$

Where, R= Result obtained from the standard curve

D.F= Dilution factor

V= Volume of stock solution

W= Weight of rice bran used

3.2.4.4. DPPH radical scavenging activity

Determination of DPPH radical scavenging activity was performed with some modification described previously [25, 29]. A portion of the extract (100 µl) solution was pipetted out in a test tube and followed by addition of 3.9 mL of methanol and 1.0 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) prepared freshly (1.0 mM) methanolic solution. The mixture was kept in the dark at an ambient temperature for 30 min and the absorbance was measured at 517 nm. The formula for calculating scavenging activity is given in Eq. (3.2).

$$\text{DPPH radical scavenging (\%)} = [1 - (\text{Absorbance}_{517\text{nm control}} / \text{Absorbance}_{517\text{ sample}})] * 100 \qquad (3.2)$$

3.2.4.5. Total anthocyanin content (TAC)

For total anthocyanin content (TAC) measurement, the optimized method of Abdel-Aal et al., [1] was incorporated with some modification. Rice bran (300 mg) was mixed in 10 mL of acidified ethanol (0.1 N HCl). The solutions were homogenized using mechanical shaker for 1 h and supernatant were collected after centrifugation at 1500 rpm. The supernatants were poured into 50 mL volumetric flask and diluted with acidified ethanol to mark. The absorbances of all the solutions were measured at 535 nm against acidified ethanol (blank) using a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA).

The total anthocyanin content of all the bran samples were determined based on the cyaniding-3-glucoside content using Lambert-Beer Law as follows (Eq. 3.3):

$$\text{TAC (mg/g)} = [(\text{Absorbance} \times \text{Extract volume} \times \text{MW} \times 10^6) / (1000 \times \varepsilon \times \text{mass})]/1000 \quad (3.3)$$

Where: ε = molar absorptivity of cyanidin-3-glucoside (25,965 L/cm mol)

MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol)

3.2.5. Cooking properties

The cooking properties were analysed using the method followed by Singh et al., [37].

3.2.5.1. Minimum cooking time

Head rice samples (2 g) were cooked in 20 mL distilled water taken in a test tube in a boiling water bath for each variety. The cooking time was determined during cooking by removing a few kernels and pressed them between two glass slides until no white core was left at different time intervals.

3.2.5.2. Elongation ratio

The length of 10 cooked rice kernels was arranged and the cumulative were measured. 10 uncooked raw kernels length was taken and the ratio was calculated. The result thus obtained was reported as an elongation ratio.

3.2.5.3. Water uptake ratio

After each head rice sample varieties had been cooked for a minimum cooking time (20 mL distilled water) in a boiling water bath, the liquids were drained off. Cooked rice samples were patted dry by pressing with filter paper. Weighing of the cooked samples was done accurately and the water uptake ratio was calculated (Eq. 3.4).

$$\text{Water Uptake Ratio} = \frac{weight\ of\ cooked\ rice}{weight\ of\ uncooked\ rice} \quad (3.4)$$

3.2.5.4. Gruel solid loss

After determining the minimum cooking time, the gruel was poured to a beaker (50 mL) with several washings and volume was made up with distilled water. The aliquot having leached solids was evaporated in an oven at 110 ^{o}C until completely dry. The solids were weighed and percent gruel solids were reported [38].

3.2.6. Texture Analysis

Textural profile analysis (TPA) of the cooked rice was performed using a Texture Analyser (TA-HD plus, Stable Micro System, UK) with a 50 kg load cell. A single layer of cooked rice samples weighing approximately about 7.5 g was placed horizontally at the center of the probe (75 mm diameter) for texture measurement. A two-cycle compression force versus time program was used to compress the samples till 70 % of the original cooked grain thickness, return to the original position and again compress. A P/75 (75 mm diameter) probe was used to compress a single layer of cooked rice samples with pre-test speed of 5.0 mm/s, test speed of 0.1 mm/s and post-test speed of 0.5 mm/s. Parameters recorded from the test curves were hardness, adhesiveness and springiness. This process was carried out in triplicate.

3.2.7. Color

The color of rice grain samples at different conditions for both cooked and uncooked rice grains were determined by CIE color scales *L**, *a** and *b** using Hunter colorimeter (Ultra Scan VIS, HunterLab, a41-1013-504, Reston, VA). Where *L** indicates the degree of lightness or darkness of the sample extended from 0 (black) to 100 (white), *a** indicates the degree of redness (+*a*) to greenness (-*a*) and *b** indicates the degree of yellowness (+*b*) to blueness (-*b*). For cooked rice color measurement, head rice (5 g) was cooked for

maximum 30 min in 30 mL distilled water. After cooking, the remaining water is drained off and the rice samples were poured it in flat filter paper, tapped the excess water with clean muslin cloth. The cooked rice was then packed in a transparent zip pouch and color measurement was performed.

3.2.8. Pasting properties

Rapid Visco Analyzer (RVA starch master 2 pulverisette instrument, Newport Scientific, Unit 1, 2 Apollo Street, Australia) was used to study the pasting properties of the whole rice flour [34]. Viscosity profiles of the flours were analysed using 2 g of samples, and 25 mL of distilled water, forming rice flour suspensions. The sample was hold initially at temperature 50-95 ^{o}C in 3.45 min, and secondly the holding phase was at 95 ^{o}C for 2.40 min. cooling phase from 95 to 50 ^{o}C in 4 min and finally the holding temperature was kept at 50 ^{o}C for 1 min. Pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity and setback were obtained.

3.2.9. Statistical Analysis

The data were analysed statistically using SPSS software (SPSS PASW 18.0) and the means were separated using Duncan's multiple range tests at a significance level of $p \leq 0.05$. One way variance analysis was carried out for obtained data to assess the significance difference among the rice varieties. All the data are presented as the mean ± SD.

3.3. Results and discussion

3.3.1. Proximate composition of pigmented rice

The proximate compositions for all the rice varieties were analysed and represented in Table 3.1. The moisture contents varied from 11.49 to 12.16 g/100g which is in line with the moisture content of China red and China black rice the values ranged between 11.90 g/100g and 11.26 g/100g respectively [39]. Protein contents for all rice varieties were found in between 5.49 to 10.66 g/100g. The protein content for purple rice seemed to appear similar content (10.4 %) as Thailand black rice [39]. The fat contents were found to lie between 1.78 to 2.94 g/100g. The protein and fat content were reported highest in purple rice. The fibre content was found to be under same range for all varieties of rice. White rice seemed to have lesser nutrient composition as compare to the colored rice

varieties. The protein, lipid, fibre and ash contents were reported to be in the range of 7.95-9.52, 2.06-2.60, 4.96-8.08 and 1.27-1.54 g/100 g respectively for brown rice samples [8].

3.3.2. Physicochemical properties

Wide variations were observed in physicochemical properties which are shown in Table 3.1. The highest thousand kernels weight was observed to be in white rice (24.59 g) followed by black (21.17 g), red (20.97 g). However, purple rice had the lowest 1000-kernel weight of 16.39 g. Thousand kernels weight do not differ significantly ($p \leq 0.05$) for black and red rice variety. Fig. 3.1 indicated the four different rice varieties used for the analysis. The average single rice grain weight was 0.022 g for black rice, 0.023 g for red, 0.019 g for purple and 0.037 g for white rice. The average length was 0.63 cm for black, 0.62 cm for red, 0.61 cm for purple and 0.65 cm for white rice. The average breadth was 0.23 cm for black rice, 0.22 cm for red, 0.20 cm for purple and 0.25 cm for white rice. The measurement was carried out using Rice Scanner (CanoScan 9000F Mark 11) machine. This can be related to the genetic basis of the pigmented rice varieties (red, black and purple) which greatly affects the quality of the rice varieties. Among all varieties, black rice showed the highest (0.65 g/cm^3) bulk density which then followed by white rice (0.61 g/cm^3), purple (0.60 g/cm^3) and red (0.59 g/cm^3). The varieties which have larger grain rice showed lower bulk densities.

Amylose content for all the different pigmented rice was found to range between 1.49 % and 6.44 % and all comes under very low amylose content. Significant differences were observed in all the rice varieties ($p \leq 0.05$). The difference in the content of amylose for all varieties may be due to different in varieties, methods of processing, areas of growing and condition of environment [42]. The lowest amylose content was found in black rice (1.49 %), and the highest in purple rice (6.44 %). Variance analysis determined that differences were significant in amylose content. Low amylose rice is soft and sticky when coked but as increase in amylose content, the rice becomes firmer [19]. For milled brown rice, an amylose content varied from 5.5–11.7 % have been represented from different cultivars [38]. Amylose content is the most important parameter that determines the rice quality. From the study, it was observed that the pigmented rice varieties were more aromatic than the white rice varieties.

Fig. 3.1. Rice Varieties (1) White rice, (2) Purple rice, (3) Red rice and (4) Black rice

Table 3.1. Proximate compositions and physicochemical properties for all the rice varieties

Varieties	Moisture (g/100g)	Crude protein (g/100g)	Crude Fat (g/100g)	Crude Fibre (g/100g)	Ash (g/100g)	Total Carbohydrate (g/100g)	1000 kernels weight (g)	Bulk density (g/cm^3)	Length (cm)	Breadth (cm)	L/B ratio	Amylose content (g/100g)
Chakhao Amubi (Black)	12.16 ± 0.24^{c}	6.16 ± 0.09^{b}	1.90 ± 0.1^{a}	2.2 ± 0.21^{a}	2.80 ± 0.11^{c}	74.78 ± 0.80^{b}	21.17 ± 0.87^{b}	0.65 ± 0.01^{b}	0.63 ± 0.01^{b}	0.23 ± 0.01^{bc}	2.87 ± 0.08^{b}	1.49 ± 0.16^{a}
Chakhao Angangba (Red)	11.93 ± 0.13^{b}	5.49 ± 0.18^{a}	2.10 ± 0.15^{b}	2.3 ± 0.1^{a}	1.40 ± 0.11^{a}	76.78 ± 0.98^{c}	20.97 ± 1.04^{b}	0.59 ± 0.02^{a}	0.62 ± 0.02^{a}	0.22 ± 0.02^{ab}	3.00 ± 0.07^{c}	2.31 ± 0.06^{a}
Chakhao Poreiton (Purple)	11.87 ± 0.40^{b}	10.66 ± 0.40^{c}	2.94 ± 0.05^{c}	2.2 ± 0.15^{a}	1.90 ± 0.16^{b}	70.43 ± 0.87^{a}	16.69 ± 0.63^{a}	0.60 ± 0.02^{a}	0.61 ± 0.01^{a}	0.20 ± 0.01^{a}	3.25 ± 0.06^{d}	6.44 ± 0.92^{c}
Chakhao Angouba (White)	11.49 ± 0.20^{a}	5.49 ± 0.20^{a}	1.78 ± 0.10^{a}	2.1 ± 0.2^{a}	1.53 ± 0.15^{a}	77.61 ± 1.07^{c}	24.59 ± 0.90^{c}	0.61 ± 0.01^{a}	0.65 ± 0.01^{c}	0.25 ± 0.01^{c}	2.68 ± 0.11^{a}	3.37 ± 0.17^{b}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p\leq0.05$)

3.3.3. Phytochemical properties

Identification of content of phytochemicals of all varieties of pigmented rice and its bran were examined and the outcomes are presented in Table 3.2. A boxplot showing the variation of phytochemical contents for both whole rice and rice bran is given in Fig. 3.2. Total phenolic content of all pigmented varieties varied significantly ($P<0.05$). It was observed that TPC ranged from 1.80 to 12.70 (mg GAE/g) of raw sample and 1.96 to 15.33 (mg GAE/g) for the bran. The highest phenolic content was recorded in black rice accompanied by purple rice which is also found similar for bran. Variations in TPC were observed in all the varieties. It was observed that the bran seemed to possess more phenolic content than the rice. Antioxidative constituents present in the plant products are being consumed and is the most effective phenolic group [45]. Black rice (12.70 mg/g) showed the highest TPC, whereas red rice (1.80 mg/g) showed the lowest value. The distribution of phenolics in grains at the cellular and subcellular levels is not uniform. Again, the content of some phenolics may increase under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures. Black and purple rice seemed to appear similar phenolic content as observed in [32]. Moreover, the phenolics level in rice grain is also dependent on various factors as cultivation techniques, rice breeding, growing conditions, ripening process as well as processing and storage conditions, among others [40].

Total flavonoid content of all the pigmented rice and bran varieties varied from 0.47 to 6.12 mg catechin/g and 0.13 to 16.45 mg catechin/g respectively. The maximum TFC was found in red rice (6.12 mg catechin /g), followed by black rice (5.45 mg/g) whereas the minimum was observed for white rice (0.47 mg catechin /g). White rice seemed to possess lowest phytochemical content than the pigmented rice. The antioxidant activities for all rice varieties were analyzed by DPPH assay. Significant difference was observed for scavenging activity towards the free radical for all rice varieties. Scavenging activity varied from 35.48 % to 92.62 % and 32.68 to 90.93 % for rice and bran respectively. Red rice showed the highest activity (92.62 %), accompanied by purple rice (82.83 %), while the lowest was observed for white rice (35.48 %). It was found that the pigmented rice showed significantly high ($p\leq0.05$) levels of polyphenols, flavonoids and antioxidant activity compared to white rice.

Total anthocyanin content was also measured using method suggested by Bulatao et al., [3] for black, red and purple rice. It was observed that total anthocyanin content was found to vary from 0.17 to 4.56 (mg Cyanidin-3-glucoside/g) while for the bran, it was observed to contain more anthocyanin content than the rice. This may be due to the whiter kernel part of the rice. The pigmented rice varieties are having color in their outer covering layers. It is only the bran that contains the color pigments. The content of anthocyanin was observed to be high in the black rice variety as compared to red and purple rice varieties.

Table 3.2. Phytochemical properties for all the rice varieties

	Varieties	TPC (mg GAE/g)	TFC (mg catechin/g)	DPPH (%)	Anthocyanin (mg Cyanidin-3-glucoside/g)
Pigmented Rice	**Chakhao Amubi (Black rice)**	12.70±0.18^{c}	5.45±0.44^{c}	67.62±2.02^{b}	4.56±0.99^{b}
	ChakhaoAngangba (Red rice)	6.58±0.19^{b}	6.12±0.77^{d}	92.62±1.82^{d}	0.17±0.01^{a}
	Chakhao Poreiton (Purple rice)	7.00±0.01^{b}	1.47±0.25^{b}	82.83±1.49^{c}	3.93±0.07^{b}
	Chakhao Angouba (White rice)	1.80±0.14^{a}	0.47±0.16^{a}	35.84±2.15^{a}	-
Pigmented Rice Bran	**Chakhao Amubi (Black rice bran)**	15.33±0.26^{c}	13.71±0.47^{c}	80.86±1.11^{b}	9.13±0.37^{b}
	Chakhao Angangba (Red rice bran)	10.62±0.11^{b}	11.22±0.43^{b}	90.93±1.07^{c}	0.35±0.06^{a}
	Chakhao Poreiton (Purple rice bran)	15.21±2.68^{c}	11.60±0.24^{b}	81.42±1.20^{b}	8.61±0.67^{b}
	Chakhao Angouba (White rice bran)	1.96±0.21^{a}	0.13±0.03^{a}	32.68±1.15^{a}	-

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$) (This is calculated separately for rice and rice bran)

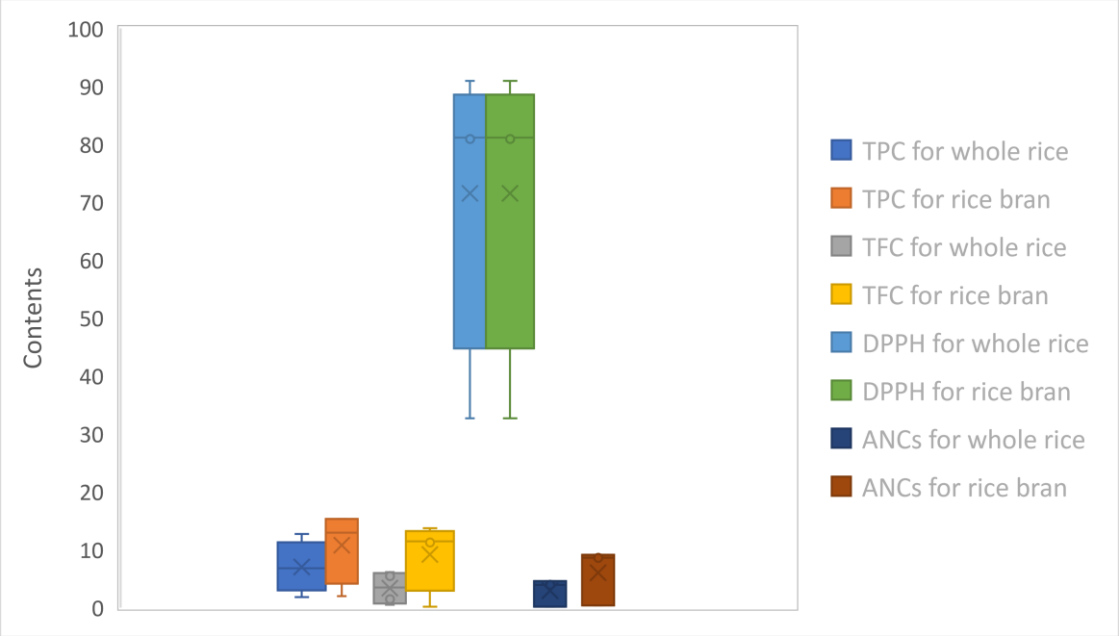

Fig. 3.2. Boxplot comparing the variation of TPC (mg GAE/g) for whole rice and rice bran, TFC (mg catechin/g) for whole rice and rice bran, DPPH (%) for whole rice and rice bran and ANCs (mg Cyanidin-3-glucoside/g) for whole rice and rice bran.

3.3.4. Cooking properties

The cooking qualities attributed by different varieties are presented in Table 3.3. The time of cooking for different pigmented rice varieties varied from 33.35 to 39.37 min. The highest cooking time (31.33 min) was observed in the purple rice which then followed by black rice (27.61 min), white rice (26.69 min) and red rice (24.37). The differences in cooking time are led by the differences in the content of amylose and the granule structure. Fig. 3.3. shows the boxplot for cooking properties. The high temperature during cooking enables crystallization process, where the interaction between the chain of long amylopectin and amylose molecule occurs. It has been extended alongside ‘cluster’ which produces two helices and creates a low swelling degree. Solids leaching and hardened texture of rice get reduced due to this crystallization process. In milled rice for fresh short and long grains, the time of cooking was observed to be in range 15–25 min in the varieties of North American [7]. The elongation ratio was found a maximum in white rice, and a minimum in purple rice which might be due to the highest amylose content which the hydrogen bond held tightly among the particles and leads to bind the molecules within themselves. This shows that the elongation ratio is positively correlated with gruel solid loss. The range of water uptake ratio was found to lie between 2.03 to 2.71, and the highest was found in white rice.

Table 3.3. Cooking characteristics and textural properties of cooked rice for all the rice varieties

Varieties	Cooking time (min)	Elongation ratio	Water uptake ratio	Gruel solid loss (%)	Hardness (N)	Adhesiveness (N/s)	Springiness
Chakhao Amubi (Black)	27.61±1.05^{b}	1.14±0.01^{ab}	2.34±0.22^{b}	8.65±0.21^{c}	58.96±5.45^{a}	5.44±2.16^{a}	0.504±0.19^{a}
Chakhao Angangba (Red)	24.37±0.96^{a}	1.15±0.01^{b}	2.71±0.15^{c}	6.87±0.15^{b}	158.26±49.52^{b}	10.47±3.73^{b}	0.568±0.19^{a}
Chakhao Poreiton (Purple)	31.33±1.17^{c}	1.13±0.01^{a}	2.15±0.20^{ab}	4.45±0.27^{a}	173.18±24.82^{b}	6.78±0.35^{ab}	0.542±0.03^{a}
Chakhao Angouba (White)	26.69±1.07^{b}	1.19±0.02^{c}	2.03±0.13^{a}	8.95±0.39^{c}	24.93±0.30^{a}	15.14±0.65^{c}	0.442±0.03^{a}

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$)

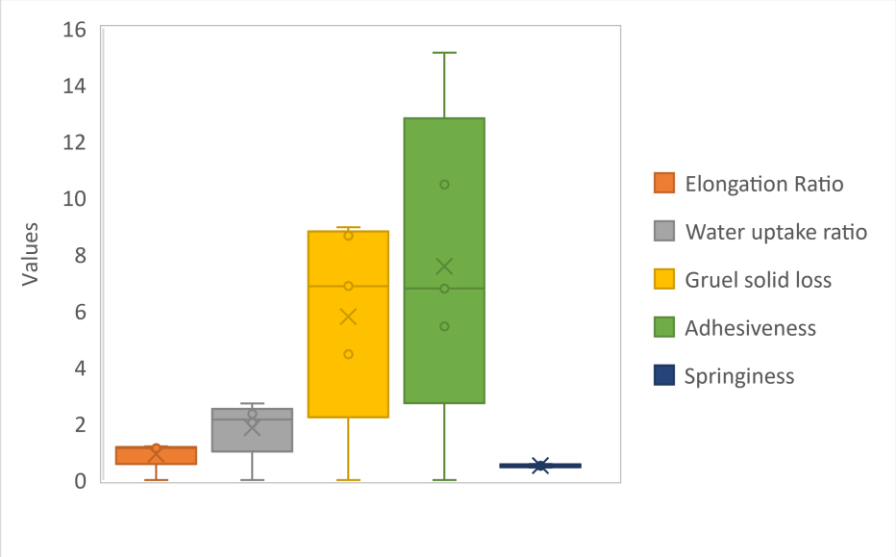

Fig. 3.3. Boxplot showing the variation of cooking and textural properties (Adhesiveness, N/s and springiness, %)

3.3.5. Texture Analysis

Texture profile analysis for cooked rice was determined for all rice varieties. Hardness value was found higher in the purple rice (173.18 N) than other rice variety which is shown in Table 3.3. High content in amylose may be the reason attributed in purple rice variety than the other three varieties. Yu et al., [46] also determined positively correlation of stiffness with the amylose contained. Significant decreases were observed in stickiness of cooked rice with higher hardness value which is formed due to leaching of high amylose content into the cooking water [18]. In a short period, amylose can easily retrograde and increases the stiffness of cooked rice. Springiness (length/length) is a measure of initial compression that breaks down the gel structure. This value ranged from 0.442 (white rice) to 0.568 (red rice) and adhesiveness from 5.44 (black rice) to 15.14 (white rice) also showed in boxplot Fig. 3.3. The variation in amylose and amylopectin led to these changes of formation of gel network during cooking. Since, the difference is found to be less in these values; red rice (0.568) possesses higher gel structure than others. During the first TPA compression, a gel structure is broken into few larger pieces appearing high springiness and vice versa.

3.3.6. Color values

It was observed that there was significant difference ($p<0.05$) in the color at different conditions for both cooked and uncooked for all the rice varieties. The values for L^*, a^* and b^* under different condition is shown in Table 3.4 (Fig. 3.4). The L^* value decreases

by cooking process in black rice from 40.04 to 24.77, in purple from 42.72 to 31.48, in red from 53.47 to 37.62 and in white from 77.20 to 54.97 respectively which means a decrease in lightness. And apart from white rice the highest *L** value was observed in uncooked red rice (53.47) followed by purple (42.72) and black (40.04) respectively and the lowest *L** value in cooked black rice (24.77). However, *a** value was found highest in cooked red rice which after cooking process got darkened in color, whereas maximum b* value was observed in uncooked red rice. During cooking or parboiling process, it was observed that there is an increase in soaking temperature which leads to decrease in lightness (*L**) and increase redness (*a**) and yellowness (*b**) of brown rice of different cultivars as reported in literature [24]. This changes in color parameters are mainly due to Maillard reaction [20]. Moreover, degradation of anthocyanins (monomeric) is caused by heat processing as well as the formation of polymers that results to browning, and is related to discoloration [17].

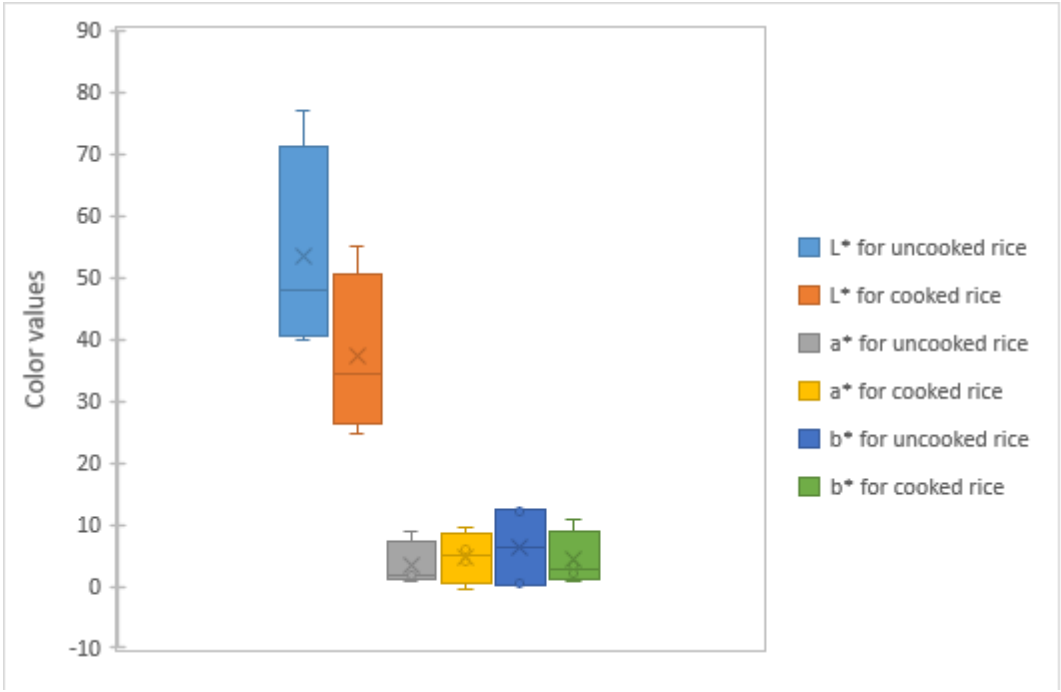

Fig. 3.4. Boxplot comparing the color values and color parameters of uncooked and cooked rice

Table 3.4. Color values for cooked and uncooked rice

Varieties	Conditions	Parameters		
		L*	***a****	***b****
Chakhao Amubi (Black)	Uncooked	40.04±0.51[a]	1.72±0.62[ab]	0.03±0.48[a]
	Cooked	24.77±0.46[a]	6.08±0.98[c]	3.35±0.47[b]
Chakhao Angangba (Red)	Uncooked	53.47±0.48[c]	8.85±0.88[c]	12.31±1.07[b]
	Cooked	37.62±1.08[c]	9.42±0.61[d]	10.94±1.10[c]
Chakhao Poreiton (Purple)	Uncooked	42.72±1.00[b]	2.05±0.22[b]	0.36 ±0.13[a]
	Cooked	31.48±3.42[b]	4.04±3.42[b]	2.05±0.51[a]
Chakhao Angouba (White)	Uncooked	77.20±1.27[d]	0.76±0.20[a]	12.28±0.41[b]
	Cooked	54.97±0.97[d]	-0.55±0.04[a]	0.95±0.12[a]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$)

3.3.7. Pasting properties

Pasting properties of all rice flour varieties are represented in Fig. 3.5 (Table 3.5). Pasting temperature is the indication of minimum temperature which is required to cook the rice flour. These lie in range between 63 to 100 ºC, the highest was found in red rice flour, and lowest for white rice. High pasting temperature indicates that the starch flour is highly resisted to swell and rupture. Peak velocity is the maximum velocity obtained by mixture when gelatinized during heat treatment in water, i.e., water-holding capacity [35]. The lowest Peak viscosity was found for black rice (854 cP) and the highest for white rice flour (2594 cP). An increase in temperature has shown a gradual increase in viscosity for all the starch flours. The removal of water by granules from the released amylose as they swell may be the reason attributed to increase in viscosity [13]. The ability of starch to form thick paste is being shown by final viscosity. Molecules variation in amylose leads to the variation in final viscosity of the samples. Mixing degree, shearing force and temperature are the factors for breakdown viscosity of any mixture [43]. The viscosity breakdown lies in the range between (Red) 4 to (white) 369. The higher the viscosity breakdown is, the ability of the starch to withstand high temperature and shear stress at the time of cooking (Tester and Morrison 1990). The value of breakdown indicated the heat stability by the

starch component at 95 °C. Hence, less breakdown value represents heat stability [15]. Setback is determined by ability of gelling or tendency of retrogradation upon cooling of cooked rice flour pastes. Setback viscosity of all rice variety varied from (purple) 382 to (black) 1682 cP. Table 3.4 shows different viscogram data for all rice flour varieties.

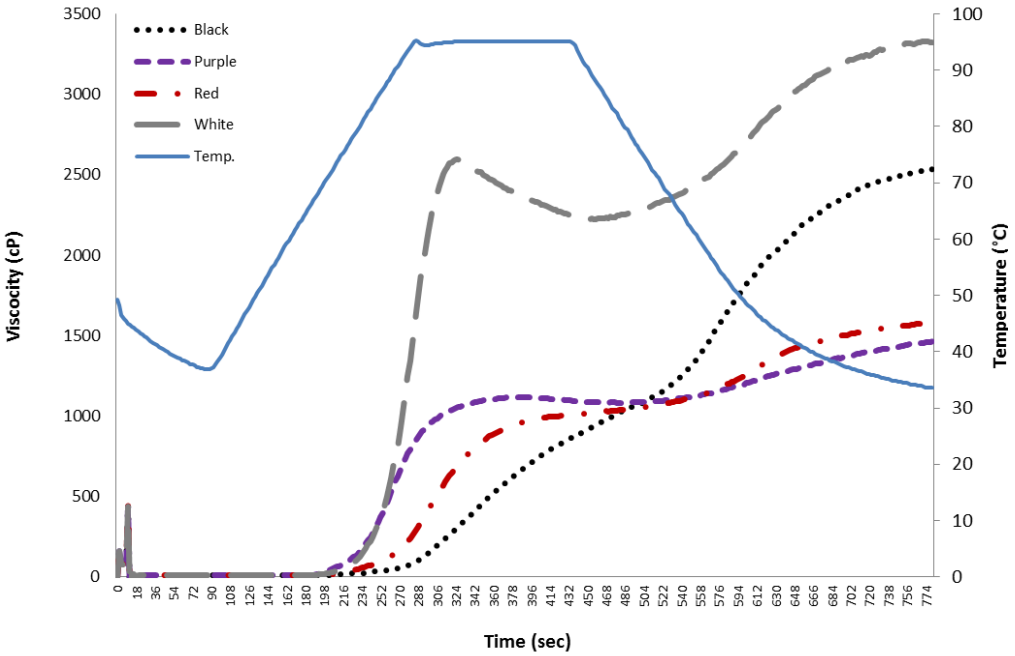

Fig. 3.5. Rapid viscoamylography of different rice cultivars

Table 3.5. Pasting properties of flours of different varieties of pigmented rice

Pasting properties	Chakhao Amubi (Black)	Chakhao Angangba (Red)	Chakhao Poreiton (Purple)	Chakhao Angouba (White)
Pasting temperature (°C)	88	100	77	63
Peak viscosity (cP)	854	1005	1118	2594
Hold viscosity (cP)	834	1001	1082	2225
Final viscosity (cP)	2536	1582	1464	3323
Break down viscosity (cP)	20	4	36	369
Set back viscosity (cP)	1682	581	382	1089

All data are the mean ± SD of three replicates.

3.4. Conclusions

Rice properties were investigated and compared with other published literature. The research work concludes that the colored rice variety has numerous medicinal and nutritional properties over white rice. Protein and fat contents were found a maximum for purple rice. From the analysis of research work, categorization of pigmented rice varieties from very low amylose content (black rice) to waxy rice (purple and red) varieties is made. There were significant differences among the different rice varieties for both physicochemical and thermal properties ($p<0.01$). Phytochemicals of pigmented rice have a potential of a bioactive compound. Polyphenols, flavonoids contents were observed higher in colored rice varieties as compared to non-pigmented rice. The content of anthocyanin was observed highest in the black rice variety. A similarity trend was also observed for phytochemical content, antioxidant activity and anthocyanin content of all the rice bran varieties. The colored rice varieties also had high antioxidant activity. Hence, there should be encouragement of consuming traditional foods of black rice, purple rice and red rice to accomplish the requirements of nutrient and for preservation of the existing food culture of the society. Moreover, colored rice can be a source of nutraceuticals as they could modify to incorporate in the formulation of therapeutic diets that improves the utilization of pigmented rice in food and non-food industries.

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